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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

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Datasheet for 100-401-264

NFkB p65 (RelA) Phospho S276 Antibody

Overview

Description:	Anti-NFKB p65 (Rel A) pS276 (RABBIT) Antibody - 100-401-264
Item No.:	100-401-264
Size:	100 µL
Applications:	ELISA, IHC, WB, IF
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	NF-kB was originally identified as a factor that binds to the immunoglobulin kappa light chain enhancer in B cells. It was subsequently found in non-B cells in an inactive cytoplasmic form consisting of NF-kB bound to IkB. NF-kB was originally identified as a heterodimeric DNA binding protein complex consisting of p65 (RelA) and p50 (NFKB1) subunits. Other identified subunits include p52 (NFKB2), cRel, and RelB. The p65, cRel, and RelB subunits are responsible for transactivation. The p50 and p52 subunits possess DNA binding activity but limited ability to transactivate. p52 has been reported to form transcriptionally active heterodimers with the NF-kB subunit p65, similar to p50/p65 heterodimers. Low levels of p52 and p50 homodimers can also exist in cells. The heterodimers of p52/p65 and p50/p65 are regulated by physical inactivation in the cytoplasm by IkB-alpha. IkB-alpha binds to the p65 subunit preventing nuclear localization and DNA binding. Activators mediate a rapid phosphorylation of IkB by IkB kinase (IKK) which results in subsequent ubiquitination and proteolytic degradation. NF-kB is then transported to the nucleus, where it activates transcription of target genes through binding to NF-kB target sequences within the promoter. The HTLV-I protein Tax can induce constitutive NF-kB activation through phosphorylation of both IkB-alpha and IkB-beta. The transforming protein Tax inhibits p53 transcriptional activity through the NFkB signaling pathway, specifically via the p65 (RelA) subunit. The inhibition of p53 activity is dependent upon phosphorylation of p65 (RelA) at S536 by the upstream kinase IKKβ. Anti-NFkB antibody is ideal for Cell Biology, Nuclear Signaling, Neuroscience and Signal Transduction Research.
Synonyms:	rabbit anti-NFkB p65 pS276 Antibody, rabbit anti-RelA pS276 Antibody, NFKB, nfkb, NF-kB, NF-kappaB, NFkappaB, Anti-NF-kB antibody
Host Species:	Rabbit
Clonality:	Polyclonal

Format:	Antiserum

Target Details

Gene Name:	RELA
Reactivity:	Human
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	NFkB p65 (Rel A) peptide corresponding to an internal region near phospho Serine 276 of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	Anti-NFkB p65 (RelA) Phospho S276 Antibody was prepared from monospecific antiserum by delipidation and defibrination. This phospho specific polyclonal antibody is specific for phosphorylated pS276 human p65. Reactivity with non-phosphorylated p65 is minimal. Cross reactivity with pS276 phosphorylated p65 from mouse, rat or other species has not been determined.
Relevant Links:	• UniProtKB - Q04206 • NCBI - 223468676 • GeneID - 5970

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	IF (Based on references)
Application Note:	Phospho NFkB antibody reacts human pS276 p65 and shows minimal reactivity by western blot with non-phosphorylated p65 and minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide. A 1:500 dilution has been used for staining p65 in human kidney tissue by IHC. Tissue was formalin fixed and paraffin embedded. Although not tested, this antibody is likely functional in immunoprecipitation. All conditions must be user optimized.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:30,000
IHC:	1:200-1:800
WB:	1:1,000 - 1:5,000

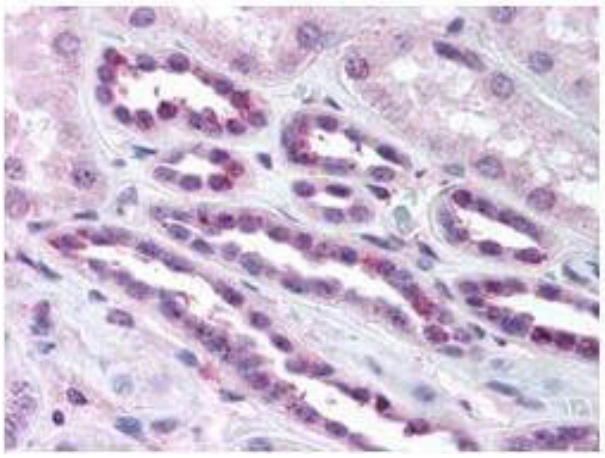
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	80 mg/mL by Refractometry
Buffer:	None
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

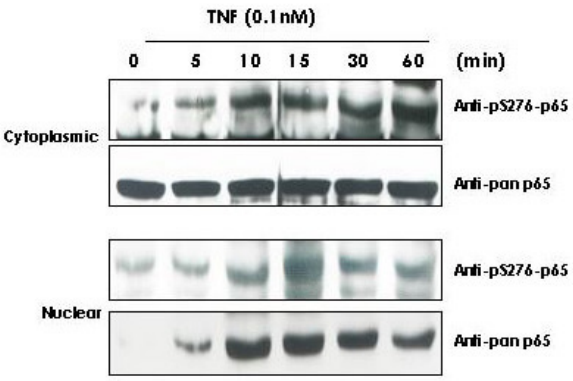
Shipping & Handling

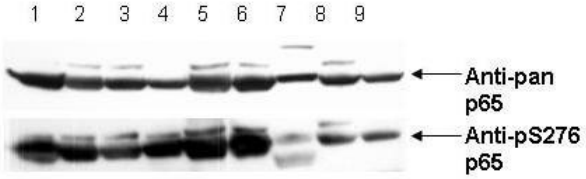
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Immunohistochemistry
Rockland's anti-p65 pS276 antibody was diluted 1:500 to detect p65 in human kidney tissue. Tissue was formalin fixed and paraffin embedded. No pre-treatment of sample was required. The image shows the localization of antibody as the precipitated red signal, with a hematoxylin purple nuclear counter stain.





Western Blot

TNF Induces phosphorylation of p65 in KBM-5 cells. Cytoplasmic and nuclear protein lysates prepared after 0, 5, 10, 15, 30 and 60 minutes of 0.1 nM TNF treatment of KBM-5 cells shows inducible phosphorylation using phospho specific polyclonal anti-human pS276 p65. Rockland Immunochemical's pan reactive anti p65 (code# 100-4165) was used a control to show the presence of total p65 in both the cytoplasmic and nuclear extracts. Phosphorylation of p65 occurs after approximately 10 min of TNF exposure. Migration of phosphorylated p65 into the nucleus occurs within a similar time frame. HRP conjugated Gt-anti-Rabbit IgG was used to develop the blot using a chemi -luminescent detection method. Other detection methods will yield similar results. Personal Communication, Aggarwal BB

Western Blot

Anti-pS276 shows phospho p65 staining in carcinoma cells. Western blot of total protein lysates from various human head and neck tumors shows phospho p65 staining in tumor cell lines using phospho specific polyclonal anti-human pS276 p65. Lanes 1-6 contain protein lysates from human squamal carcinoma cell lines. Lane 7 is a protein lysate from a primary culture of human keratinocytes and does not show significant levels of phosphorylated p65. Lane 8 contains protein lysate from ATCC SCC9 cells (also a head and neck squamal carcinoma). Lane 9 contains lysate from EGF-induced human derived A431 cells. Lane 10 (not shown) contains a molecular weight standard. Concurrent staining with anti-beta microtubulin (not shown) was used to confirm equal protein loading in all lanes. HRP conjugated Gt-anti-Rabbit IgG was used to develop the blot using a chemiluminescent detection method. Other detection methods will yield similar results. Data contributed by Yu, M., NIH, personal communication.

References

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